The Noyes Chemical Laboratory, University of Illinois

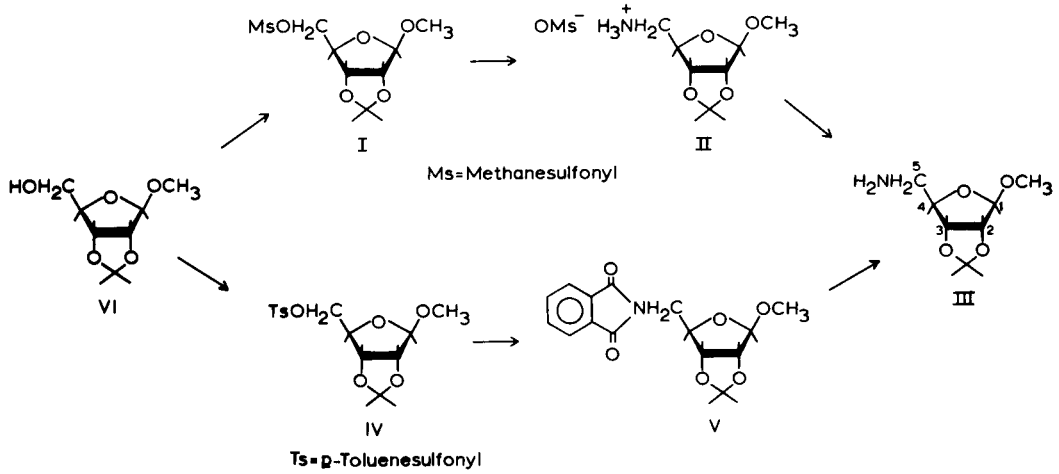
5-Amino-5-deoxyribose Derivatives. Synthesis and Use in the Preparation of "Reversed" Nucleosides (1a)

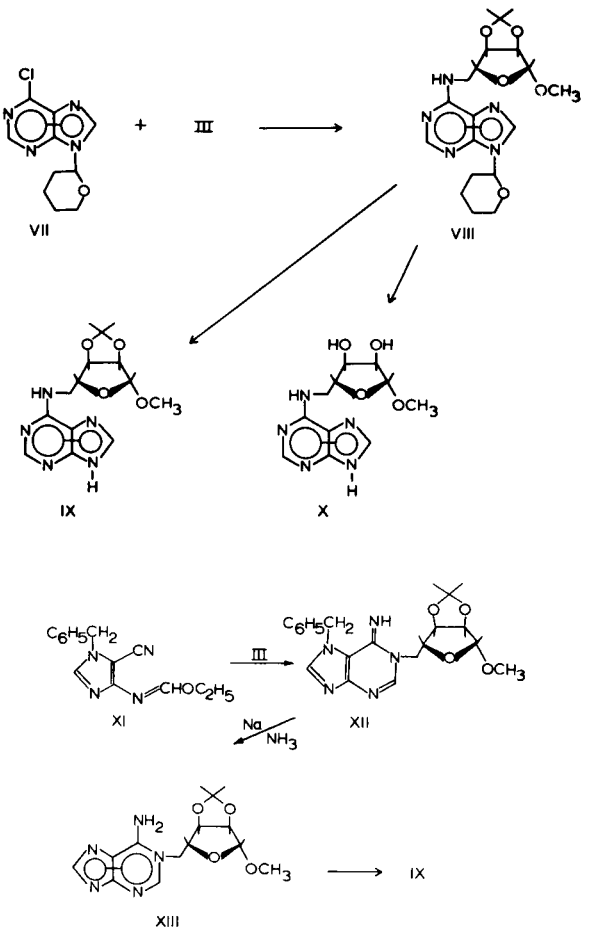
Nelson J. Leonard and Kermit L. Carraway (1b)

Methyl 5-amino-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (III) has been synthesized and used as an intermediate in the preparation of the kinetin analog, methyl 5-deoxy-5-(purin-6-yl)amino-β-D-ribofuranoside (X). The related 1-substituted adenine, methyl 5-(6-aminopurin-1-yl)-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (XIII), was prepared by cyclization of 1-benzyl-5-cyano-4-ethoxymethyleneaminoimidazole (XI) with III and subsequent debenzylation with sodium in liquid ammonia. The structures and stereochemistry of these compounds were established by a combination of ultraviolet and nuclear magnetic resonance spectroscopy.

As part of a cooperative program with Professor Skoog at the University of Wisconsin (2a) to determine the cytokinin activity (2b,3) and chemical properties of compounds closely related to kinetin (6-furfurylaminopurine) (4,5), a route to ribose or deoxyribose derivatives of adenine bonded at C-5 of the sugar ("reversed" nucleosides) was sought. To coincide with other synthetic work on nucleoside derivatives, routes to N^6- and to 1-substituted derivatives were emphasized. It was decided that the simplest approach to these compounds would be through the 5-aminoribose derivatives.

Baker and Kissman (6) had reported the synthesis of "very impure" methyl 5-amino-5-deoxy-2,3-*O*-isopropylidene-D-ribofuranoside (III) by displacement on the mesylate (I) with ammonia and subsequent liberation of the base (II → III) by ion exchange. It seemed likely that a more promising route to III might be found via the phthalimide derivative V, which had also been synthesized by Baker and Kissman. Although the essential features of this route were established previously, some comment seems desirable concerning the stereochemistry of the intermediates. The original preparation of methyl 2,3-*O*-isopropylidene-D-ribofuranoside (VI) by Levene and Stiller (7) was assumed to be a mixture of anomers although there was no evidence to support or disprove this assumption. Reist and coworkers (8) assigned a β configuration to IV without derivation of the assignment. No attempt was made to infer a β configuration for VI from these results. As recently as 1964, however, Montgomery and Hewson (9) assumed a mixture of anomers from the preparation of VI and accordingly used a more laborious route (10) to obtain a pure β isomer. The nmr spectra of methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (VI), methyl 2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribofuranoside (IV), methyl 5-deoxy-2,3-*O*-isopropylidene-5-phthalimido-β-D-ribofuranoside (V), and methyl 5-amino-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (III) show conclusively, on the basis of the singlet signal (at 60 Mc) for H_1 (11-18), that for each of these the assignment of the β configuration is justified. Although the synthetic procedure for obtaining VI is slightly modified from the original, the isolation is





similar enough to indicate that the same product is obtained.

Treatment of the phthalimide intermediate V (6) with hydrazine in methanol gave the free amine III, which was purified through its hydrochloride for analysis. This purification was not necessary for carrying out later reactions of the amine. Reaction of the amine with 6-chloro-9-(2-tetrahydropyranyl)-purine (VII) (19) in dioxane yielded methyl 5-deoxy-2,3-*O*-isopropylidene-5-[9-(2-tetrahydropyranyl)-purin-6-yl]amino-β-D-ribofuranoside (VIII). The 9-substituted 6-chloropurine VII was used because of its greater solubility and reactivity (19). The use of the tetrahydropyranyl blocking group is indicated when substitution by highly sensitive amines at the 6-position is intended. We found, moreover, that the tetrahydropyranyl group could be removed selectively from VIII (⟶ IX) by mild treatment with acid in methanol-acetone. Both the tetrahydropyranyl and isopropylidene groups could be removed from VIII (⟶ X), without altering the anomeric configuration, by treatment with hydrochloric acid in refluxing methanol.

Confirmation of the structural assignments of methyl 5-deoxy-2,3-*O*-isopropylidene-5-[9-(2-tetrahydropyranyl)purin-6-yl]amino-β-D-ribofuranoside (VIII), methyl 5-deoxy-2,3-*O*-isopropylidene-5-(purin-6-yl)amino-β-D-ribofuranoside (IX), and methyl 5-deoxy-5-(purin-6-yl)amino-β-D-ribofuranoside (X) was obtained from their microanalytical, ultraviolet and nmr data. The ultraviolet spectra of VIII are typical of an N^6,9-dialkyladenine (20), and those of IX and X are typical of a 6-alkylaminopurine. The nmr spectrum (60 Mc) for each compound indicated, by the signal for the C_1 proton (singlet for VIII and IX, doublet, J = 1 cps, for X), that each possessed the β configuration.

Methyl 5-deoxy-5-(purin-6-yl)amino-β-D-ribofuranoside (X) (21) shows cytokinin activity in the tobacco bioassay (22). The minimal detectable growth-promoting activity of tobacco callus tissue was found at a concentration of 0.1-0.5 μM/l, and the level for optimal growth, at 2.5 μM/l. The corresponding figures for kinetin were within the ranges 0.0003-0.004 μM/l and 0.1-0.7 μM/l, respectively, in parallel growing periods. Details of the activity of this compound and others in the series are reported elsewhere (2a).

Analogous 1-substituted adenines can be obtained from an imidazole precursor via the route of Taylor and Loeffler (23), which was followed for the preparation of 7-benzyladenine and 1,7-dibenzyladenine (20) and has been applied more recently to the preparation of 7-β-D-ribofuranosyladenine (24). The combination of 1-benzyl-5-cyano-4-ethoxymethyleneaminoimidazole (XI) with methyl 5-amino-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (III) yielded methyl 5-[7-benzyl-6(1*H*)-iminopurin-1-yl]-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (XII). The benzyl group was readily removed by treatment of XII in suspension in liquid ammonia with sodium to give methyl 5-(6-aminopurin-1-yl)-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (XIII). The structures of XII and XIII were assigned on the basis of microanalyses and ultraviolet absorption spectra, which were typical of 1,7-dialkyl and 1-alkyl substitution, respectively. Compound XIII underwent conversion on melting to N^6-substituted product, as in the rearrangement of 1-benzyladenine to 6-benzylaminopurine (25).

The debenzylation step in the synthesis described above merits some discussion. Hydrogenolysis using palladium on charcoal is one method which has been used for the debenzylation of substituted adenines, exemplified in the synthesis of 7-benzyladenine (26), 7-methyladenine (26), 1-methyladenine (27), and 7-ribofuranosyladenine (28). The selective hydrogenolysis of 3,7-dibenzyladenine hydrochloride to 7-benzyladenine suggests preferential cleavage of a benzyl group attached to the pyrimidine ring of adenine (26). In corroboration, we have found that the major product of hydrogenolysis of 1,9-dibenzyladenine and 1,7-dibenzyladenine is in each case that resulting from removal of the 1-benzyl group.

A second method of debenzylation was considered on the basis of Boissonnas' review (29) of amine protecting groups, which indicating that *N*-benzylimidazole groups are removed slowly by catalytic hydrogenation but readily by sodium in liquid am-

monia. Beyond the removal of *O*-benzyl groups from 9-(2',3',5'-tri-*O*-benzyl-β-D-arabinofuranosyl)-adenine (30), which was accomplished by sodium in liquid ammonia (31), and the statement that the removal of the benzyl group from the N-3 position of hypoxanthines was difficult with sodium in liquid ammonia (28), the technique has seen little use in purine chemistry, in contrast to its broad and successful application in peptide chemistry (29, 32-34). The general stability of the adenine ring system (31, 35) should permit these reduction conditions to be used, but the possibility of rearrangement (25) or the occurrence of other reactions of 1-substituted adenines had to be considered. Accordingly, before we applied sodium in liquid ammonia to the debenzylation of XII we performed the reduction of 7-benzyladenine (36), 7-benzyl-1-ethyladenine (37), and 7-benzyl-6-ethylaminopurine (37). The respective products: adenine, 1-ethyladenine, and 6-ethylaminopurine, were identified by direct comparison in the first case and by microanalytical data, ultraviolet spectra, and paper chromatography (37,38) in the other two cases. These results suggest that the sodium-ammonia method of *N*-debenzylation is capable of more general application in the purine series.

EXPERIMENTAL

Methyl 2,3-*O*-Isopropylidene-β-D-ribofuranoside (VI) (7).

A solution of 50 g. (0.33 mole) of dry D-ribose in 1.0 l. of acetone, 100 ml. of 2,2-dimethoxypropane and 200 ml. of methanol containing 20 ml. of methanol saturated with hydrogen chloride at 0° was stirred at 25° overnight. The resulting orange solution was neutralized with pyridine and evaporated to a yellow oil. This oil was partitioned between 500 ml. of water and 200 ml. of ether. The water layer was extracted twice with 200 ml. portions of ether, and the combined ether extracts were dried. Evaporation yielded a pale yellow oil which was distilled at 0.3 mm. and 75° to give 47 g. (70%) of the colorless, protected riboside, n_D^{24} 1.4507, $[\alpha]_D^{25}$ -82.2° (2% in chloroform).

The nmr spectrum ($CDCl_3$) showed signals at τ values 5.00 (1H, singlet, H_1), 5.15, 5.29 (2H, AB pair, J = 6.3 cps, H_2, H_3), 5.59 (1H, triplet, J = 3.7 cps, H_4), 6.33 (2H, doublet, J = 3.7 cps, $2H_5$), 6.56 (3H, singlet, OCH_3), 6.80 (1H, singlet, OH), 8.52 (3H, singlet, CCH_3), 8.68 (3H, singlet, CCH_3).

Methyl 2,3-*O*-Isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribofuranoside (IV) (39).

This compound was prepared by the method of Levene and Stiller, m.p. 83-84° (lit. 83-84°), in 78% yield. The nmr spectrum ($CDCl_3$) showed signals at τ values 2.17, 2.63 (4H, AB pair, J = 8 cps, aromatic), 5.06 (1H, singlet, H_1), 5.3-6.1 (5H, multiplet, H_2, H_3, H_4, $2H_5$), 6.80 (3H, singlet, OCH_3), 7.58 (3H, singlet, tosyl CH_3), 8.60 (3H, singlet, CCH_3), 8.75 (3H, singlet, CCH_3).

Methyl 5-Deoxy-2,3-*O*-isopropylidene-5-phthalimido-β-D-ribofuranoside (V) (6).

A suspension of 10.0 g. (28.0 mmoles) of methyl 2,3-*O*-isopropylidene-5-*O*-*p* = toluenesulfonyl-β-D-ribofuranoside (IV), 6.7 g. (36.0 mmoles) of potassium phthalimide and 0.8 g. of sodium iodide was heated at about 150° in 200 ml. of dimethylformamide for 20 minutes. The resulting solution was cooled and poured into 1.0 l. of ice water. The colorless crystals which formed were collected by filtration and washed with water to yield 7.8 g. (84%) of product, m.p. 128-130°. An analytical sample was prepared by recrystallization from methanol as colorless plates, m.p. 128-128.5°, $[\alpha]_D^{25}$ -50.1° (2.7% in chloroform) [lit. (6) 128-128.5°, $[\alpha]_D^{25}$ -45.2 (2.5% chloroform)].

The nmr spectrum ($CDCl_3$) showed signals at τ values 2.0-2.2 (4H, multiplet, aromatic), 4.95 (1H, singlet, H_1), 5.1-6.2 (5H, multiplet, H_2, H_3, H_4, $2H_5$), 6.62 (3H, singlet, OCH_3), 8.58 (3H, singlet, C-CH_3), 8.73 (3H, singlet, C-CH_3).

Anal. Calcd. for $C_{17}H_{19}NO_8$: C, 61.25; H, 5.74; N, 4.20. Found: C, 60.95; H, 5.70; N, 3.99.

Methyl 5-Amino-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (III) and its Hydrochloride.

A solution of 2.5 g. (7.5 mmoles) of methyl 5-deoxy-2,3-*O*-isopropylidene-5-phthalimido-β-D-ribofuranoside (V) and 0.42 g. (11.0 mmoles) of 85% hydrazine hydrate in 20 ml. of methanol was heated under reflux for 2 hours. The methanol was removed *in vacuo* from the resulting suspension, and the white solid residue was dissolved in 40 ml. of water and acidified to *p*H 1. The precipitate of phthalhydrazide was removed by filtration, and the colorless filtrate was brought to *p*H>12. The basic solution was extracted three times with 30 ml. of chloroform and the extracts were dried. Evaporation of the chloroform gave 1.3 g. (85%) of the colorless liquid amine. The amine could be purified for analysis by conversion to its hydrochloride. An ethereal solution of the amine was treated with a saturated solution of hydrogen chloride in ether. The resulting precipitate was recrystallized from absolute ethanol as dense, colorless needles, m.p. 201.5-202°, $[\alpha]_D^{25}$ -34.5° (6.3% in water). The nmr spectrum (D_2O) of the hydrochloride showed signals at τ values 4.34 (1H, singlet, H_1), 4.61 (2H, multiplet, H_2, H_3), 4.79 (HDO), 5.04 (1H, triplet, H_4), 5.98 (3H, singlet, OCH_3), 6.1-6.4 (2H, multiplet, $2H_5$), 7.98 (3H, singlet, CCH_3), 8.12 (3H, singlet, CCH_3).

Anal. Calcd. for $C_9H_{18}ClNO_4$: C, 45.09; H, 7.57; N, 5.85. Found: C, 45.28; H, 7.49; N, 5.66.

The pure amine was obtained from an aqueous solution of the hydrochloride, which had been brought to *p*H>12, by extraction with chloroform. Evaporation of the chloroform gave a colorless oil, $[\alpha]_D^{25}$ -71.6° (0.8% in chloroform). The nmr spectrum ($CDCl_3$) showed signals at τ values 5.11 (1H, singlet, H_1), 5.3-5.5 (2H, multiplet, H_2, H_3), 5.91 (1H, triplet, H_4), 6.56 (3H, singlet, OCH_3), 7.28 (2H, doublet, $2H_5$), 8.5-8.7 (2H, unresolved, NH_2), 8.57 (3H, singlet, CCH_3), 8.73 (3H, singlet, CCH_3).

Anal. Calcd. for $C_9H_{17}NO_4$: C, 53.19; H, 8.43; N, 6.89. Found: C, 53.48; H, 8.45; N, 6.98.

Methyl 5-Deoxy-2,3-*O*-isopropylidene-5-[9-(2-tetrahydropyranyl)purin-6-yl]amino-β-D-ribofuranoside (VIII).

To the colorless amine obtained from 6.5 g. (19.5 mmoles) of methyl 5-deoxy-2,3-*O*-isopropylidene-5-phthalimido-β-D-ribofuranoside (V) as described above was added 1.5 g. (7.4 mmoles) of 6-chloro-9-(2-tetrahydropyranyl)purine (VII) (19) and 10 ml. of dioxane. The solution was stirred at 60° overnight. The resultant semisolid was filtered with the aid of dioxane to yield the solid amine hydrochloride. The filtrate was evaporated to a slightly yellow oil, which crystallized from ether, 1.7 g., m.p. 148-150°. Partition of the crude hydrochloride between water and chloroform, and further extraction of the aqueous layer with chloroform gave an additional 0.3 g. of product, m.p. 146-149° (total yield 78%). Recrystallization from ether gave colorless prisms, m.p. 148-150°, $[\alpha]_D^{25}$ -29.1° (3.2% in chloroform).

The ultraviolet spectra showed the following: 0.1*N* HCl in 95% ethanol, unstable; 95% ethanol, λ max 265.5 mμ (ε, 17,100), 210 (19,700), λ min 229 (1,950); 0.1*N* NaOH in 95% ethanol, λ max 265.5 (18,000), λ min 238.5 (6,450). The nmr spectrum ($CDCl_3$) showed signals at τ values 1.70 (1H, singlet, purine), 1.95 (1H, singlet, purine), 3.3-3.5 (1H, multiplet, NH), 4.1-4.3 (1H, multiplet, tetrahydropyranyl CH), 4.96 (1H, singlet, H_1'), 5.31 (2H, multiplet, H_2', H_3'), 5.45 (1H, triplet, H_4'), 5.8-6.3 (4H, multiplet, $2H_5'$ and tetrahydropyranyl CH_2), 6.56 (3H, singlet, OCH_3), 7.7-8.1 (4H, multiplet, tetrahydropyranyl CH_2), 8.1-8.4 (2H, multiplet, tetrahydropyranyl CH_2), 8.53 (3H, singlet, CCH_3), 8.72 (3H, singlet, CCH_3).

Anal. Calcd. for $C_{19}H_{27}N_5O_5$: C, 56.27; H, 6.71; N, 17.27. Found: C, 56.37; H, 6.72; N, 17.35.

Methyl 5-Deoxy-2,3-*O*-isopropylidene-5-(purin-6-yl)amino-β-D-ribofuranoside (IX).

A solution of 369 mg. (0.92 mmole) of methyl 5-deoxy-2,3-*O*-isopropylidene-5-[9-(2-tetrahydropyranyl)purin-6-yl]amino-β-D-ribofuranoside (VIII) in 30 ml. of a 1:1 methanol-acetone mixture containing 0.1 ml. of concentrated sulfuric acid was allowed to stand 4 hours at 25°. The solution was neutralized with 2*N* sodium hydroxide and evaporated to a gum. This was dissolved in water and extracted three times with 15-ml. portions of chloroform. Evaporation of the combined extracts gave a white solid, which was washed with ether and filtered to yield 120 mg. (41%), m.p. 212-214°, $[\alpha]_D^{25}$ -31.9° (0.7% in ethanol). Recrystallization from ethyl acetate produced the analytical sample as fiber-like crystals, m.p. 213-215°.

The ultraviolet spectra showed the following: 0.1*N* HCl in 95%

ethanol, λ max 280 mμ (ε, 17,400), λ min 237.5 (2,900); 95% ethanol, λ max 268 (19,100), λ min 229 (1,400); 0.1*N* NaOH in 95% ethanol, λ max 275.5 (13,900), λ min 241 (3,200), λ sh 279 (10,200). The nmr spectrum ($CDCl_3$) showed signals at τ values −2.8 (1H, multiplet, N^9H), 1.53 (1H, singlet, purine), 1.95 (1H, singlet, purine), 2.9-3.3 (1H, multiplet, N^6H), 4.94 (1H, singlet, H_1'), 5.1-5.6 (3H, multiplet, H_2', H_3', H_4'), 5.9-6.2 (2H, multiplet, $2H_5'$), 6.55 (3H, singlet, OCH_3), 8.52 (3H, singlet, CCH_3), 8.70 (3H, singlet, CCH_3).

Anal. Calcd. for $C_{14}H_{19}N_5O_4$: C, 52.33; H, 5.97; N, 21.80. Found: C, 52.34; H, 5.85; N, 21.76.

Methyl 5-Deoxy-5-(purin-6-yl)amino-β-D-ribofuranoside (X).

To a solution of 1.02 g. (2.5 mmoles) of methyl 5-deoxy-2,3-*O*-isopropylidene-5-[9-(2-tetrahydropyranyl)purin-6-yl]amino-β-D-ribofuranoside (VIII) in 25 ml. of methanol was added 2.5 ml. of 2*N* hydrochloric acid, and the solution was refluxed for 1 hour. The methanol was partially removed *in vacuo* and the solution was diluted to 25 ml. with water. The aqueous solution was neutralized with Dowex-1 (HCO_3^-). Filtration of the resin and evaporation of the filtrate gave a yellow solid, which was recrystallized from ethanol, 159 mg., m.p. 236-238°, $[\alpha]_D^{25}$ −22.5° (0.4% in ethanol). Evaporation of the ethanol yielded 329 mg. of identical material (total yield 69%).

The ultraviolet spectra showed the following: 0.1*N* HCl in 95% ethanol, λ max 278 mμ (16,400), λ min 237 (3,050); 95% ethanol, λ max 268 (16,700), λ min 227 (1,300); 0.1*N* NaOH in 95% ethanol, λ max 275.5 (16,800), λ min 237.5 (4,700), λ sh 284 (12,300). The nmr spectrum (D_2O) showed signals at τ values 1.55 (1H, singlet, purine), 1.59 (1H, singlet, purine), 4.62 (1H, doublet, J = 1 cps, H_1'), 4.75 (HDO), 5.4-5.6 (2H, multiplet, H_2', H_3'), 5.45 (1H, triplet, H_4'), 5.9-6.2 (2H, multiplet, $2H_5'$), 6.34 (3H, singlet, OCH_3).

Anal. Calcd. for $C_{11}H_{15}N_5O_4$: C, 46.96; H, 5.37; N, 24.91. Found: C, 46.71; H, 5.36; N, 24.62.

Methyl 5-[7-Benzyl-6(1*H*)-iminopurin-1-yl]-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (XII).

Methyl 5-amino-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (III) was prepared from 1.9 g. (5.7 mmoles) of the corresponding phthalimide (V). The amine was dissolved in 20 ml. of ethanol, and 0.58 g. (2.3 mmoles) of 1-benzyl-5-cyano-4-ethoxymethyleneaminoimidazole (XI) (20) was added. The solution was refluxed overnight, cooled and evaporated to a yellow oil, which crystallized upon the addition of ether. The colorless solid was filtered and washed with ether to give 0.77 g. (82%), m.p. 135-139°. Recrystallization from ethyl acetate gave the analytical sample as fluffy, colorless needles, m.p. 139-143°, $[\alpha]_D^{25}$ −80.6 (1% in chloroform).

The ultraviolet spectra showed the following: 0.1*N* HCl in 95% ethanol, λ max 278 mμ (ε, 7,800), 222 (24,800), λ min 248 (5,300); 95% ethanol, λ max 265 (9,300), λ min 238 (2,100); 0.1*N* NaOH in 95% ethanol, λ max 265 (10,000), λ min 241 (4,100). The nmr spectrum ($CDCl_3$) showed signals at τ values 2.34 (2H, singlet, purine), 2.5-3.0 (5H, multiplet, aromatic), 3.5-3.9 (1H, broad singlet, NH), 4.54 (2H, singlet, benzyl CH_2), 5.04 (1H, singlet, H_1'), 5.2-6.4 (3H, multiplet, H_2', H_3', H_4'), 6.64 (3H, singlet, OCH_3), 8.65 (3H, singlet, CCH_3), 8.73 (3H, singlet, CCH_3).

Anal. Calcd. for $C_{21}H_{25}N_5O_4$: C, 61.29; H, 6.13; N, 17.02. Found: C, 61.50; H, 6.18; N, 16.93.

Methyl 5-(6-Aminopurin-1-yl)-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (XIII).

To a stirred suspension of 411 mg. (1 mmole) of methyl 5-[7-benzyl-6(1*H*)-iminopurin-1-yl]-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (XII) in 150 ml. of liquid ammonia was added sodium metal until the blue color persisted. Ammonium chloride was added to discharge the color, and the ammonia was allowed to evaporate. The residue was dissolved in ethanol-water and brought to *p*H 7 with 2*N* hydrochloric acid. The neutral solution was evaporated to a tan residue, which was dissolved in ethanol and filtered to remove some inorganic salts. The remaining material was chromatographed on 60 g. of silica gel using methanol-ethyl acetate as an eluent. The product was eluted at a concentration of 30% methanol (800 ml.). Evaporation yielded 267 mg. (79% as monohydrate) of white powder, homogeneous by thin layer chromatography. Recrystallization from acetone gave the analytical sample as a hygroscopic, microcrystalline monohydrate.

The ultraviolet spectra showed the following: 0.1*N* HCl in 95% ethanol, λ max 259 mμ (ε, 11,800), λ min 233 (2,300); 95% ethanol, λ max 271 (11,100), 227 (18,100); λ min 244 (3,800); 0.1*N* NaOH in 95% ethanol, λ max 273 (12,800), 268 (12,500), λ min 242 (3,000).

Anal. Calcd. for $C_{14}H_{19}N_5O_4 \cdot H_2O$: C, 49.54; H, 6.24; N, 20.64. Found (dried at room temperature): C, 49.68; H, 6.04; N, 19.99. Calcd. for $C_{14}H_{19}N_5O_4$: N, 21.79. Found (dried at 100°, 24 hours): N, 21.72.

This compound shows unusual melting point behavior. A gradual phase change, beginning at 130°, occurs as the temperature is raised. Darkening begins at 190° and continues until the final melting with decomposition at 230°. The ultraviolet spectrum of the sample (after melting or during the process) indicates that rearrangement occurs. Maxima are observed at 266 mμ (neutral), 275 (acidic), and 275, 283 (sh) (basic), indicating that the product is N^6-substituted, probably mainly IX.

Hydrogenolysis of 1,9-Dibenzyladenine.

A solution of 225 mg. (0.71 mmole) of 1,9-dibenzyladenine (20) in 100 ml. of ethanol was shaken for 2 hours at room temperature at 2.5 atmospheres of hydrogen with 100 mg. of 10% palladium on carbon. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was washed with ether and filtered to give 123 mg. (77%) of 9-benzyladenine as colorless needles, m.p. 232-234°, identified by mixing melting point and ultraviolet spectral comparisons (20,36).

Anal. Calcd. for $C_{12}H_{11}N_5$: C, 63.98; H, 4.93; N, 31.09. Found: C, 63.91; H, 4.94; N, 30.81.

Hydrogenolysis of 1,7-Dibenzyladenine.

A solution of 0.20 g. (0.63 mmoles) of 1,7-dibenzyladenine (20) in 100 ml. of ethanol was shaken with 0.10 g. of 10% palladium on charcoal under 2 atmospheres of hydrogen for 18 hours at 25°. The catalyst was removed by filtration, and the solution was evaporated to dryness. The residue was washed with ether and filtered to give 0.08 g. (56%) of 7-benzyladenine, which was contaminated with a trace of residual charcoal. Recrystallization from ethanol-water gave pure material, m.p. 235-237°, which was identical with an authentic sample by mixture melting point and ultraviolet spectroscopic comparisons (26,36).

Debenzylation of 7-Benzyladenine.

To a stirred suspension of 265 mg. (1.2 mmoles) of 7-benzyladenine (20,26) in 50 ml. of liquid ammonia was added sodium metal in small portions until the characteristic deep blue color persisted. The color was discharged by the addition of 200 mg. of ammonium chloride, and the ammonia was allowed to evaporate. The resulting residue was washed with benzene and dissolved in 2*N* hydrochloric acid. Neutralization and evaporation gave a white residue, which was washed with water and filtered to yield 116 mg. (73%) of a colorless powder, m.p. > 300°, homogeneneous by thin layer chromatography. This material was identified as adenine by direct comparison of infrared, ultraviolet and thin layer chromatographic characteristics with those of an authentic sample.

REFERENCES

(1a) Supported in part by a research grant (USPHS-GM 05829, currently -08) from the National Institutes of Health, U. S. Public Health Service, to whom we are pleased to acknowledge our thanks; (b) National Science Foundation Predoctoral Fellow, 1963-1966.

(2a) F. Skoog, H. Q. Hamzi, A. Szweykowska, N. J. Leonard, K. L. Carraway, T. Fujii, J. P. Helgeson, and R. N. Loeppky, manuscript in preparation; (b) F. Skoog, F. M. Strong, and C. O. Miller, *Science*, 148, 532 (1965).

(3) J. van Overbeek, *ibid.*, 152, 721 (1966).

(4) F. M. Strong, "Topics in Microbial Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1958.

(5) C. O. Miller, *Ann. Rev. Plant Physiol.*, 12, 395 (1961).

(6) B. R. Baker and H. M. Kissman (to American Cyanamid Co.), U. S. Patent 2,875,194, February 24, 1959; *Chem. Abstr.*, 53, 13072 h (1959).

(7) P. A. Levene and E. T. Stiller, *J. Biol. Chem.*, 104, 299 (1934).

(8) E. J. Reist, R. R. Spencer, M. E. Wain, I. G. Junga, L. Goodman, and B. R. Baker, *J. Org. Chem.*, 26, 2821 (1961).

(9) J. A. Montgomery and K. Hewson, *ibid.*, 29, 3436 (1964).

(10) R. Barker and H. G. Fletcher, Jr., *ibid.*, 26, 4605 (1961).

(11) M. Karplus, *J. Chem. Phys.*, 30, 11 (1959); *J. Am. Chem. Soc.*, 85, 2870 (1963).

(12) R. U. Lemieux and J. W. Lohn, *Can. J. Chem.*, 41, 889 (1963).

(13) L. Goldman, J. W. Marsico, and M. J. Weiss, *J. Med. Chem.*, 6, 410 (1963).

(14) L. Goldman and J. W. Marsico, *ibid.*, 6, 413 (1963).

(15) N. J. Leonard and R. A. Laursen, *J. Am. Chem. Soc.*, 85, 2026 (1963).

(16) N. J. Leonard and R. A. Laursen, *Biochemistry*, 4, 354 (1965).
(17) C. D. Jardetzky, *J. Am. Chem. Soc.*, 84, 62 (1962).
(18) L. D. Hall, *Adv. Carbohydrate Chem.*, 19, 51 (1964).
(19) R. K. Robins, E. F. Godefroi, E. C. Taylor, L. R. Lewis, and A. Jackson, *J. Am. Chem. Soc.*, 83, 2574 (1961).
(20) N. J. Leonard, K. L. Carraway, and J. P. Helgeson, *J. Heterocyclic Chem.*, 2, 291 (1965).
(21) Professor E. Lederer has kindly informed us of the synthesis in his laboratory of a 6-(dihydroxytetrahydrofurfurylamino)purine, more closely related to kinetin in oxidation state.
(22) T. Murashige and F. Skoog, *Physiol. Plantarum*, 15, 473 (1962).
(23) E. C. Taylor and P. K. Loeffler, *J. Am. Chem. Soc.*, 82, 3147 (1960).
(24) R. J. Rousseau, L. B. Townsend and R. K. Robins, *Chem. Commun.*, 265 (1966).
(25) N. J. Leonard, S. Achmatowicz, R. N. Loeppky, K. L. Carraway, W. A. H. Grimm, A. Szweykowska, H. Q. Hamzi, and F. Skoog, *Proc. Natl. Acad. Sciences*, 56, 709 (1966).
(26) N. J. Leonard and T. Fujii, *J. Am. Chem. Soc.*, 85, 3719 (1963).
(27) N. J. Leonard and T. Fujii, *Proc. Natl. Acad. Sciences*, 51, 73 (1964).
(28) J. A. Montgomery and H. J. Thomas, *J. Am. Chem. Soc.*, 87, 5442 (1965).
(29) R. A. Boissonnas, *Adv. Org. Chem.*, 3, 159 (1963).
(30) C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Org. Chem.*, 28, 3004 (1963).
(31) E. J. Reist, V. J. Bartuska, and L. Goodman, *ibid.*, 29, 3725 (1964).
(32) V. du Vigneaud and O. K. Behrens, *J. Biol. Chem.*, 117, 27 (1937).
(33) E. Schnabel and C. H. Li, *J. Am. Chem. Soc.*, 82, 4576 (1960).
(34) G. C. Stelakatos and N. Argyropoulos, *Chem. Commun.*, 270 (1966).
(35) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience, New York, N. Y., 1963, pp. 210-211.
(36) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, 83, 630 (1961).
(37) K. L. Carraway, Ph.D. Thesis, University of Illinois, 1966.
(38) B. C. Pal, *Biochemistry*, 1, 558 (1962).
(39) P. A. Levene and E. T. Stiller, *J. Biol. Chem.*, 106, 421 (1934).

Received August 15, 1966 Urbana, Illinois 61801